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(71) Applicant: TREGA BIOSCIENCES, INC. [US/US]; 3550 General Atomics Court, San Diego, CA 92121 (US).

(72) Inventors: MANIAR, Manoj; 10953 Waterton Road, San Diego, CA 92131 (US). GIRTEN, Beverly, E.; Apartment 111, 5220 Fiore Terrace, San Diego, CA 92122 (US).

(74) Agents: FAN, Calvin, A. et al.; Campbell & Flores LLP, Suite 700, 4370 La Jolla Village Drive, San Diego, CA 92122 (US).

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(54) Title: COMPOSITIONS OF THERAPEUTIC AGENTS SUITABLE FOR ORAL ADMINISTRATION

(57) Abstract

The present invention provides compositions useful for formulating a biologically active substance for oral administration. For example, the invention provides compositions comprising a microemulsion and an adjuvant such as salicylic acid.

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COMPOSITIONS OF THERAPEUTIC AGENTS SUITABLE FOR ORAL ADMINISTRATION

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates generally to the field of drug delivery and, more specifically, to compositions of therapeutic agents that can administered orally to a subject.

BACKGROUND INFORMATION

The ability to isolate biologically active substances in large amounts has provided accessability to agents that potentially can be useful as therapeutics. In addition, the ability to screen large libraries of synthetic molecules such as peptides has made available new drugs that can be directed to specific targets known to be involved in disease. Unfortunately, while such biologically active substances can show a specific effect when examined using *in vitro* assays, the same substances often are not useful when administered to an individual. In particular, potential protein or peptide drugs often are not effective when administered orally due, for example, to enzymatic degradation in the digestive tract or to lack of absorption from the intestinal lumen.

Efforts to modify such biologically active substances have been successful to various degrees. For example, peptides potentially useful as drugs have been modified by incorporating (D)-amino acids in place of one or more corresponding naturally occurring (L)-amino acids in a peptide. Such modified peptides often can be resistant to enzymatic degradation due to steroselectivity of digestive enzymes. However, such

modifications that alter the stereochemistry of a peptide also can result in the peptide not interacting with its biological target and, therefore, losing its efficacy.

In order to avoid problems associated with chemical modification of a potential biologically active substance, such substances have been formulated into compositions that physically protect the agent from degradation or improve the absorption of the agent from the gut into the circulation. Although such compositions have found use in making various biologically active substances orally available, more effective compositions continually are being sought. Thus, a need exists for compositions that permit oral administration of a biologically active substance that otherwise lacks efficacy when administered orally. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides compositions of biologically active substances that are suitable for oral administration. For example, the invention provides compositions comprising a microemulsion and a cytokine regulatory agent having the structure X_4-X_5-(D)Phe-Arg-(D)Trp-X_3 or X_4-X_5-(D)Phe-Arg-(D)Trp-X_3, where X_1, X_2, X_3, X_4 and X_5 are amino acids or amino acid analogs, or modified forms of such structures.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 compares the effect of orally administered CRA-1 with indomethacin on arachidonic acid induced ear swelling and demonstrates the dose dependent effect obtained with orally administered CRA-1.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compositions useful for formulating a biologically active substance such that the substance can be administered as an oral medicament. In particular, a composition of the invention comprises a microemulsion and an adjuvant. The invention is exemplified by the preparation of a composition comprising a microemulsion containing salicylic acid as an adjuvant, the composition further comprising the cytokine regulatory agent (CRA), Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2 ("CRA-1"). CRA's are known in the art and described, for example, in U.S. Patent No. 5,420,109; issued May 30, 1995, which is incorporated herein by reference (CRA's previously were known as "cytokine restraining agents").

As used herein, the term "microemulsion" has its commonly understood meaning of a liquid dispersion of water and oil made homogenous, transparent and stable by addition of a surfactant and a cosurfactant (see Gennaro, "Remington's Pharmaceutical Sciences" (Mack Publishing Co. 1990, which is incorporated herein by reference). In general, a microemulsion contains oil globules dispersed in the aqueous phase or water globules dispersed in the oil phase. The size of the globules generally ranges from about 10 nm to about 100 nm.

Microemulsions have been formed, for example, by dispersing an anionic surfactant such as sodium lauryl sulfate in benzene, then adding a small amount of water followed by gradual addition of a cosurfactant such as pentanol. Microemulsions are used, for example, in cosmetics, foods, dry cleaning agents, and waxes and polishes. A microemulsion useful in the invention is exemplified herein by a Formulation of "LABRAFAC LIPOPHILE WL 1349" (medium chain triglycerides) as the

oil, "PLUROL OLEIQUE CC 497" (polyglyceryl oleate FCC) as the surfactant, and "LABRASOL" (satureated polyglycolyzed C_5-C_{10} glycerides) as the cosurfactant (Gattefosse; Westwood NJ; see Example I).

As used herein, the term "adjuvant" means an agent that enhances the bioavailability of a biologically active substance. For example, an adjuvant can result in increased absorbability of the biologically active substance from the gastrointestinal tract into the circulation or can prevent the nonspecific binding of the substance so as to increase the effective concentration of the substance in a subject. An adjuvant can act, for example, by complexing with a biologically active substance, thereby enhancing the solubility of the substance. Thus, caffeine has been used an adjuvant in combination with benzocaine to enhance the dissolution of benzocaine. Similarly, hydroquinone has been used as an adjuvant in combination with digoxin to enhance dissolution of digoxin.

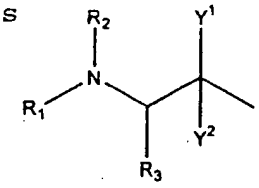
As disclosed herein, salicylic acid (SIGMA Chemical Co.; St. Louis MO) is another adjuvant, which is useful in the present invention (see Example I). Although the mechanism by which salicylic acid acts as an adjuvant, it does not appear to effect absorption of the examined biologically active substance (see Example II).

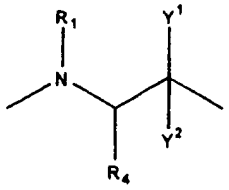
As used herein, the term "biologically active substance" means a chemical or biological molecule that is useful as a therapeutic agent. Thus, a biologically active substance can be, for example, an organic molecule or can be a peptide, polypeptide or protein. The usefulness of the claimed composition is demonstrated by the oral administration of a CRA, which is a modified peptide that is not therapeutically effective when administered orally in a free form.

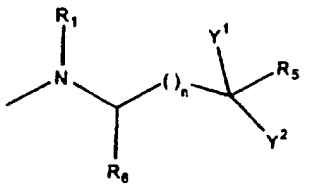
In particular, the effectiveness of the claimed composition for permitting oral delivery of a biologically active substance was demonstrated by showing that a CRA having the structure Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2 ("CRA-1"), when formulated in a composition of the invention, effectively regulates lipopolysaccharide (LPS) induced interleukin-10 ("IL-10") and tumor necrosis factor-α ("TNFα") levels and effectively reduces arachidonic acid induced dermal swelling in an experimental animal model (see Example II and Figure 1). Remarkably, the therapeutic effect of orally administered CRA-1 formulated in a composition of the invention occurred in the absence of a significant increase in plasma CRA-1 levels.

The efficacy of a composition of the invention in providing a vehicle that allows oral administration of a biologically active substance was demonstrated using a cytokine regulatory agent (CRA). In general, a CRA has the structure:

$X_1 - X_2 -His - (D)Phe - Arg - (D)Trp - X_3$, where

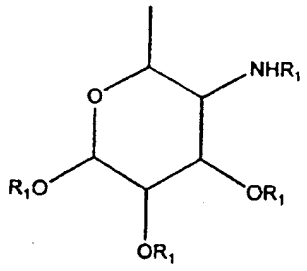
X_1 is  , H or $COCH_3$;

X_2 is  ; and

X_3 is  or R_5;

where Y^1 and Y^2 are independently a hydrogen atom, or are taken together to form a carbonyl or thiocarbonyl; R_1 is H, $COCH_3$, C_2H_5, CH_2Ph, COPh, COO-t-butyl, $COOCH_2Ph$, CH_2CO-(polyethylene glycol) or A; R_2 is H or $COCH_3$; R_3 is a linear or branched alkyl group having 1 to 6 carbon atoms or a cyclic alkyl group having 3 to 6 carbon atoms; R_4 is $(CH_2)_m$-$CONH_2$, $(CH_2)_m$-$CONHR_1$ or $(CH_2)_m$-CONHA; R_5 is OH, OR_3, NH_2, SH, $NHCH_3$, $NHCH_2Ph$ or A; and R_6 is H or R_3;

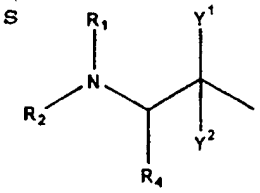
and where "Ph" is C_6H_5, "m" is 1, 2 or 3, "n" is 0, 1, 2 or 3, and "A" is a carbohydrate having the general formula:



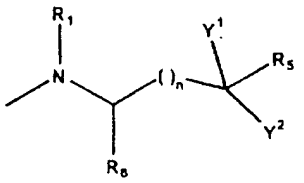
(U.S. Patent No. 5,420,109; *supra*, 1995).

In addition, a CRA can have the structure:

X_4 - X_5 - (D)Phe - Arg - (D)Trp - X_3, where

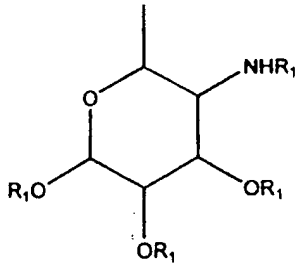
X_4 is  , H, $COCH_3$ or absent;

X_5 is His, H or $COCH_3$; and

X_3 is  , NH_2 or OH;

where Y^1 and Y^2 are independently a hydrogen atom, or are taken together to form a carbonyl or thiocarbonyl; R_1 is H, $COCH_3$, C_2H_5, CH_2Ph, COPh, COO-t-butyl, $COOCH_2Ph$, CH_2CO-(polyethylene glycol) or A; R_2 is H or $COCH_3$; R_4 is $(CH_2)_m$-$CONH_2$, $(CH_2)_m$-$CONHR_1$ or $(CH_2)_m$-CONHA; R_5 is OH, OR_3,, NH_2, SH, $NHCH_3$, $NHCH_2Ph$ or A; and R_6 is H or R_3;

and where "Ph" is C_6H_5, "m" is 1, 2 or 3, "n" is 0, 1, 2 or 3, and "A" is a carbohydrate having the general formula



(see U.S. Patent No. 5,420,109, *supra*, 1995, which also discloses methods for making a CRA).

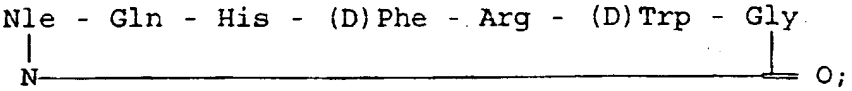
In general, a CRA is a peptide or a peptide-like structure such as a peptidomimetic or a peptoid (see Ecker and Crooke, Biotechnology 13:351-360 (1995), and Blondelle et al., Trends Anal. Chem. 14:83-92 (1995), and the references cited therein, each of which is incorporated herein by reference). Amino acids are indicated herein by their commonly known three letter code, where "(D)" designates an amino acid having the "D" configuration, as compared to the naturally occurring (L)-amino acids; "Nle" is the three letter code for norleucine. Where no specific configuration is indicated, one skilled in the art would understand the amino acid to be an (L)-amino acid. In the CRA structures shown above, "Ph" indicates a "phenyl" group (C_6H_5). CRA peptides are written in the conventional manner, such that the amino-terminus (N-terminus) is shown to the left and the carboxy-terminus (C-terminus) is shown to the right.

One skilled in the art would know that the choice of amino acids or amino acid analogs incorporated into the peptide will depend, in part, on the specific physical, chemical or biological characteristics required of the CRA. Selective modification of a reactive group in a peptide also can impart desirable characteristics to a CRA. For example, the N-terminus can be modified by acetylation or the C-terminus can be modified by amidation. Methods for modifying the N-terminus or C-terminus of a peptide are well known in the art (see, for example, in U.S. Patent No. 5,420,109, *supra*, 1995). The choice of modifications made to the reactive groups present on the peptide is determined by a desirable characteristic required in the CRA. CRA-1, which has the structure Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2, is an

example of a CRA that is modified both by acetylation at the N-terminus and by amidation at the C-terminus.

A cyclic peptide also can be an effective CRA. A cyclic peptide can be obtained by inducing the formation of a covalent bond between, for example, the amino group at the N-terminus of the peptide and the carboxyl group at the C-terminus. For example, the peptide, cyclo(His-(D)Phe-Arg-(D)Trp), can be produced by inducing the formation of a covalent bond between His and (D)Trp. Alternatively, a cyclic peptide can be obtained by forming a covalent bond between a terminal reactive group and a reactive amino acid side chain or between two reactive amino acid side chains such as the sulfhydryl reactive groups present in cysteine residues. One skilled in the art would know that the choice of a particular cyclic peptide is determined by the reactive groups present on the peptide as well as the desired characteristic of the peptide. Cyclization of a CRA peptide can provide the CRA with increased stability *in vivo*.

In addition to the examples provided above, other representative cytokine regulatory agents include:

1) Ac-Nle - Gln - His - (D)Phe - Arg - (D)Trp -Gly-OH;
2) Ac-Nle - Gln - His - (D)Phe - Arg - (D)Trp -Gly-OC_2H_5;
3) Ac-Nle - Gln - His - (D)Phe - Arg - (D)Trp -Gly-NH-NH_2;
4) Ac-Nle - Asn - His - (D)Phe - Arg - (D)Trp -Gly-NH_2;
5) Ac-Nle - Asn - His - (D)Phe - Arg - (D)Trp -Gly-OH;
6) Ac-Nle - Gln - His - (D)Phe - Arg - (D)Trp - Gly-$NHCH_2CH_2Ph$;
7) Ac-Nle - Gln - His - (D)Phe - Arg - (D)Trp - Gly-$NHCH_2Ph$;
8) 
9) Ac-Gln - His - (D)Phe - Arg - (D)Trp - Gly-NH_2;
10) Ac-Nle - Gln - His - (D)Phe - Arg - (D)Trp-NH_2;

11) His-(D)Phe-Arg-(D)Trp-NH_2;

12) Ac-His - (D)Phe - Arg - (D)Trp-OH;

13) Ac-His - (D)Phe - Arg - (D)Trp - Gly-NH_2; and

14) Ac-His-(D)Phe-Arg-(D)Trp-(CH_2NHAc)-Gly-NH_2,

where "-(CH_2NHAc)-" indicates a modified peptide bond between (D)Trp and Gly.

Peptide cytokine regulatory agents as described above are characterized, in part, by a core structure (D)Phe-Arg-(D)Trp, where the amino acids are indicated by their commonly known three letter code and where "(D)" designates an amino acid having the "D" configuration, as opposed to the naturally occurring L-amino acids. Where no specific configuration is indicated, one skilled in the art would understand the amino acid to be an (L)-amino acid. In the peptides exemplified above, "Nle" is the three letter code for norleucine and "Ph" indicates a "phenyl" group (C_6H_5).

Cytokine regulatory agents are synthesized using a modification of the solid phase peptide synthesis method of Merrifield (J. Am. Chem. Soc., 85:2149 (1964), which is incorporated herein by reference; see U.S. Patent No. 5,420,109, *supra*, 1995) or can be synthesized using standard solution methods well known in the art (see, for example, Bodanszky, M., Principles of Peptide Synthesis 2nd revised ed. (Springer-Verlag, 1988 and 1993), which is incorporated herein by reference). Peptides prepared by the method of Merrifield can be synthesized using an automated peptide synthesizer such as the Applied Biosystems 431A-01 Peptide Synthesizer (Mountain View, CA) or using the manual peptide synthesis technique described by Houghten, Proc. Natl. Acad. Sci., *USA* 82:5131 (1985), which is incorporated herein by reference.

CRA-1 was synthesized using amino acids or amino acid analogs, the active groups of which were protected as required using, for example, a t-butyldicarbonate (t-BOC) group or a fluorenylmethoxy carbonyl (FMOC) group. Amino acids and amino acid analogs can be purchased commercially (Sigma Chemical Co.; Advanced Chemtec) or synthesized using methods known in the art. Peptides synthesized using the solid phase method can be attached to resins including 4-methylbenzhydrylamine (MBHA), 4-(oxymethyl)-phenyl acetamido methyl and 4-(hydroxymethyl)phenoxymethyl-copoly(styrene-1% divinylbenzene) (Wang resin), all of which are commercially available, or to p-nitro benzophenone oxime polymer (oxime resin), which can be synthesized as described by De Grado and Kaiser, J. Org. Chem. 47:3258 (1982), which is incorporated herein by reference (see Example I).

One skilled in the art would know that the choice of amino acids or amino acid analogs incorporated into the peptide will depend, in part, on the specific physical, chemical or biological characteristics required of the cytokine regulatory peptide. The skilled artisan further would recognize that selective modification of a peptide such as a CRA can impart desirable characteristics such as increased solubility on a CRA.

With regard to selective modification of the reactive groups in a peptide, the peptides can be manipulated while still attached to the resin to obtain N-terminal modified compounds such as an acetylated peptide or can be removed from the resin using hydrogen fluoride or an equivalent cleaving reagent and then modified. Compounds synthesized containing the C-terminal carboxy group (Wang resin) can be modified after cleavage from the resin or, in some cases, prior to solution phase synthesis. Methods for modifying the

N-terminus or C-terminus of a peptide are well known in the art and include, for example, methods for acetylation of the N-terminus or methods for amidation of the C-terminus. Similarly, methods for modifying side chains of the amino acids or amino acid analogs are well known to those skilled in the art of peptide synthesis. The choice of modifications made to the reactive groups present on the peptide will be determined by the characteristics that the skilled artisan requires in the peptide.

A newly synthesized peptide can be purified using a method such as reverse phase high performance liquid chromatography (RP-HPLC; see U.S. Patent No. 5,420,109, *supra*, 1995) or other methods of separation based on the size or charge of the peptide. Furthermore, the purified peptide can be characterized using these and other well known methods such as amino acid analysis and mass spectrometry.

A composition of the invention, which comprises a microemulsion and an adjuvant, that contains a biologically active substance such as a CRA also can contain an additional material such as a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known in the art and include aqueous solutions such as physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil or injectable organic esters.

In addition, a composition of the invention can contain a physiologically acceptable compound that acts, for example, to stabilize the biologically active substance or increase the absorption of the substance. Such physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or

glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. One skilled in the art would know that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the particular physico-chemical characteristics of the specific biologically active substance.

The concentration of a biologically active substance required for a therapeutic effect will depend, for example, on the particular substance and on the disease to be treated. For example, CRA's are known to be useful for treating various conditions associated with altered cytokine activity (see U.S. Patent No. 5,420,109, *supra*, 1995). Thus, CRA's are useful for treating, for example, inflammatory reactions and patho-immunogenic diseases such as rheumatoid arthritis.

In order to effectively treat a condition characterized, in part, by altered cytokine activity, a CRA must be administered in an effective dose, which is about 0.01 to 200 mg/kg body weight per administration. The total treatment dose can be administered to a subject as a single dose or can be administered as a series of multiple doses over a period of time. One skilled in the art would know that the amount of a CRA required to obtain an effective dose in a subject depends on many factors including the specific CRA being administered and the age and general health of the subject. In view of these factors, the skilled artisan would recognize that the amount of a biologically active substance required to obtain an effective dose for treating a particular condition can be determined by monitoring the treated patient's clinical course using routine methods such as radiologic, immunologic and, where indicated, histopathologic methods.

The efficacy of using a composition of the invention for orally administering a biologically active substance was confirmed by demonstrating that oral administration of CRA-1 can regulate cytokine activity in mice exposed to bacterial lipopolysaccharide (LPS) and can decrease the amount of dermal swelling in mice treated with arachidonic acid. These animal models are recognized as useful models for bacterial sepsis and inflammation, respectively (see Inflammation 17:723-741 (1993); Am. J. Pathol. 143:1121-1130 (1993), each which is incorporated herein by reference).

The following examples are intended to illustrate but not limit the invention.

EXAMPLE I

PREPARATION OF A COMPOSITION COMPRISING A MICROEMULSION AND AN ADJUVANT

This example describes methods for preparing a composition of the invention, which permits oral administration of a CRA that otherwise is not therapeutically effective when administered orally.

A cytokine regulatory agent having the amino acid sequence Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2 (CRA-1) was prepared as described in U.S. Patent No. 5,420,109 (*supra*, 1995). A stock solution of 60 mg/ml salicylic acid in water was prepared.

Twenty grams of a mixture of 8.429 g "LABRASOL," 3.571 g "PLUROL OLEIQUE CC 497" and 8.000 g "LABRAFAC LIPOPHILE" (Gattefosse) was prepared and vortexed to a homogeneous, clear solution ("premicroemulsion"). A 6 mg/ml stock salicylic acid/microemulsion was prepared by transferring 5 g

premicroemulsion into a clean glass vial, adding 500 µl 60 mg/ml salicylic acid and vortexing until the solution was clear.

A formulation of 27 mg/ml CRA-1 in 2 mg/ml salicylic acid/microemulsion was prepared by transferring 70.0 mg CRA-1 into a clean glass vial, adding 300 µl water and vortexing and sonicating until the CRA-1 was dissolved, then transferring 190 µl of the CRA-1 solution to a clean vial containing 0.996 g premicroemulsion, vortexing the mixture until clear, adding 0.496 g 6 mg/ml salicylic acid/microemulsion and vortexing until a clear, single phase microemulsion was obtained.

A formulation of 10 mg/ml CRA-1 in 2 mg/ml salicylic acid/microemulsion was prepared by transferring 39.1 mg CRA-1 into a clean glass vial, adding 300 µl water and vortexing and sonicating until the CRA-1 was dissolved, then transferring 250 µl of the CRA-1 solution to a clean vial containing 1.992 g premicroemulsion, vortexing the mixture until clear, adding 1.008 g 6 mg/ml salicylic acid/microemulsion and vortexing until a clear, single phase microemulsion was obtained.

A "placebo" vehicle containing 2 mg/ml salicylic acid was prepared by adding 1.337 g premicroemulsion, 200 µl water and 0.675 g 6 mg/ml salicylic acid together and vortexing until the solution was clear.

EXAMPLE II

ORAL ADMINISTRATION OF CRA-1 FORMULATED IN A MICROEMULSION AND ADJUVANT IS THERAPEUTICALLY EFFECTIVE

This example compares the plasma levels of CRA-1 following intravenous injection or oral administration and demonstrates that CRA-1 formulated in a composition of the invention is therapeutically effective when administered orally.

A. Plasma concentration of CRA-1

Plasma concentrations of CRA-1 were examined following intravenous injection of free CRA-1 or oral administration of various concentrations of CRA-1 formulated in a composition of the invention (4 mice/group). Plasma CRA-1 concentrations were determined by a scintillation proximity assay based radioimmunoassay using a Packard Tri-Carb 1099 TR scintillation counter (Packard Instruments; Downers Grove IL).

Mice injected intravenously with 13.5 mg/kg CRA-1 (approximately 270 μg/mouse) had a level of about 65,000 ng CRA-1/ml blood plasma 5 min after injection. This level decreased to about 8000 ng/ml one hr after injection and essentially was a zero by 4 hr.

In comparison, mice that received 13.5 mg/kg CRA-1, which was formulated in a microemulsion and salicylic acid, by oral administration attained a maximum level of only about 600 ng/ml 10 min after administration. This level decreased to about 100 ng/ml after 1.5 hr and essentially was at zero after about 2.5 hr. In a second group of mice that received oral administration of 26.9 mg/kg CRA-1, a maximum plasma level of about 1750 ng CRA-1/ml plasma was reached by

10 min. This level decreased to about 250 ng/ml after 1.5 hr and essentially was at zero after 1.5 hr.

These results indicate that oral administration of a biologically active substance such as CRA-1 in a composition comprising a microemulsion and an adjuvant such as salicylic acid results in about a 100 fold lower plasma level of the substance as compared to intravenous administration of the substance.

B. Effect of orally administered CRA-1 on TNF and IL-10 levels following treatment with LPS

The effectiveness of CRA-1 administered orally or for decreasing tumor necrosis factor (TNF) levels and for increasing IL-10 levels in lipopolysaccharide (LPS; endotoxin) treated mice was examined.

Balb/c female mice weighing approximately 20 g were placed into six groups of eight mice each, except as indicated, as follows: two control groups, one of which was injected intraperitoneally (ip) with phosphate buffered saline (PBS) and other of which received placebo (2 mg/ml salicylic acid in microemulsion) orally; one group treated by ip injection of 300 μg CRA-1; and four groups, each of which received orally administered 0.25 mg, 0.5 mg, 1.0 mg or 2.7 mg CRA-1 formulated in microemulsion/salicylic acid (see Example I). One minute after administration of CRA-1, 100 μg LPS in 0.9% saline was administered by ip injection into the mice. In addition, a seventh group of five mice was orally administered 1.0 mg CRA-1, then LPS injection was delayed for 15 min.

Blood samples were collected from the orbital sinus of the mice 90 min after LPS was administered. The plasma was separated by centrifugation at 3000 x g for 5

min, then diluted with four volumes of 1x phosphate buffer saline (pH 7.4) containing 1% bovine serum albumin. 100 µl samples of serum were assayed by ELISA using commercially available kits for TNFα (Genzyme; Cambridge MA) or for IL-10 (R & D Systems; Minneapolis MN).

The mean (+/- SEM) TNFα and IL-10 levels were determined for each group of mice and the percent of control (PBS) for TNF and IL-10 levels were calculated. TNFα and IL-10 levels in mice injected with placebo microemulsion were essentially identical with the control (PBS) mice. Mice injected ip with 300 µg CRA-1 had TNFα levels that were about 25% of control mice and IL-10 levels that were about 325% of control. These results indicate that orally administered CRA-1 can regulate cytokine levels in LPS treated mice.

C. Effect of Orally Administered CRA-1 on Arachidonic Acid-Induced Dermal Swelling in Mice

The effect of orally administered CRA-1 on arachidonic acid-induced dermal swelling in mice was examined.

Experiments were performed using female Balb/c mice weighing approximately 20 g. Saline (control; 10 mice), indomethacin (50 mg/kg; 5 mice) or 25 (10 mice), 50 (10 mice) or 135 (9 mice) mg/kg CRA-1 in microemulsion and salicylic acid were administered orally to the mice. Thirty min later, a 10 µl pipet was used to apply 10 µl arachidonic acid (AA) solution (100 mg/ml ethanol; Calbiochem-Novabiochem; San Diego CA) to the inner and outer surfaces of the right ear of each mouse.

Ear thickness was measured with a hand-held spring loaded caliper immediately before and 60 min after

AA application. Increase in ear thickness was calculated by subtracting the ear thickness prior to AA administration from the thickness 60 min after AA administration. 50 mg/kg indomethacin reduced dermal swelling to about 40% of the swelling in control mice (Figure 1). In comparison, orally administered CRA-1 at 25, 50 or 135 mg/kg reduced swelling to about 82%, 60% or 24% of the swelling in control mice (Figure 1). These results indicate that orally administered CRA-1 reduces arachidonic acid induced ear swelling in a dose dependent manner, despite the observation that oral administration of CRA-1 did not produce a correspondingly high plasma concentration of the agent.

Although the invention has been described with reference to the examples provided above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

We claim:

1. A composition, comprising a microemulsion and an adjuvant.

2. The composition of claim 1, wherein said microemulsion comprises a formulation of "LABRASOL," PLUROL OLEIQUE CC 497" and "LABRAFAC LIPOPHILE WL 1349."

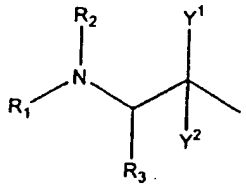
3 The composition of claim 1, wherein said adjuvant is salicylic acid.

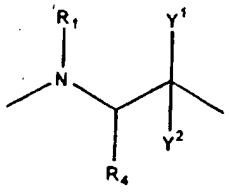
4 The composition of claim 1, further comprising a biologically active substance.

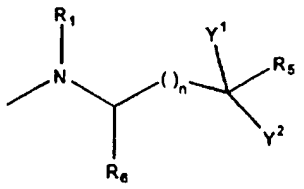
5. The composition of claim 4, wherein said biologically active substance is a cytokine regulatory agent (CRA) having the structure:

X_1 - X_2 - His - (D)Phe - Arg - (D)Trp - X_3,

wherein:

X_1 is  , H or $COCH_3$;

X_2 is  ; and

X_3 is  or R_5;

wherein Y^1 and Y^2 are independently a hydrogen atom, or are taken together to form a carbonyl or thiocarbonyl;

R_1 is H, $COCH_3$, C_2H_5, CH_2Ph, COPh, $COOCH_2Ph$, COO-t-butyl, CH_2CO-(polyethylene glycol) or A;

R_2 is H, C_2H_5, CH_2Ph or $COCH_3$;

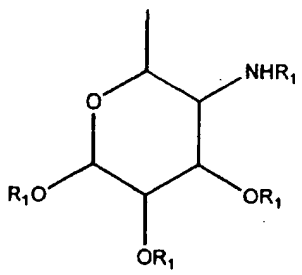
R_3 is a linear or branched alkyl group having 1 to 6 carbon atoms or a cyclic alkyl group having 3 to 6 carbon atoms;

R_4 is $(CH_2)_m$-$CONH_2$, $(CH_2)_m$-$CONHR_1$ or $(CH_2)_m$-CONHA;

R_5 is OH, OR_3, NH_2, SH, $NHCH_3$, $NHCH_2Ph$ or A; and

R_6 is H or R_3;

and wherein "Ph" is C_6H_5, "m" is 1, 2 or 3, "n" is 0, 1, 2 or 3, and "A" is a carbohydrate having the general formula:



6. The composition of claim 5, wherein the amino terminus of said CRA is modified by acetylation.

7. The composition of claim 5, wherein the carboxy terminus of said CRA is modified by amidation.

8. The composition of claim 5, wherein R_1 is selected from the group consisting of C_2H_5 and CH_2Ph and wherein R_2 is selected from the group consisting of H and $COCH_3$.

9. The composition of claim 5, wherein R_1 and R_2 are the same moiety, said moiety selected from the group consisting of H, C_2H_5 and CH_2Ph.

10. The composition of claim 5, wherein X_1 is selected from the group consisting of norleucine, norvaline, leucine or isoleucine.

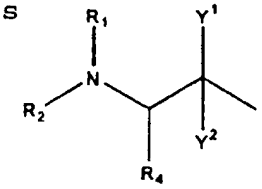
11. The composition of claim 5, wherein R_5 is covalently bound to X_1, said covalent bond forming a cyclic peptide.

12. The composition of claim 4, wherein said biologically active substance is a CRA having the structure:
Gln - His - (D)Phe - Arg - (D)Trp - Gly-NH_2.

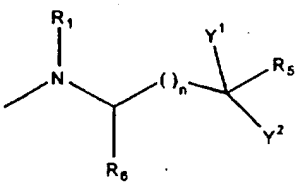
13. The composition of claim 12, wherein the amino terminus of said CRA is acetylated.

14. The composition of claim 4, wherein said biologically active substance is a CRA having the structure:

X_4 - X_5 - (D)Phe - Arg - (D)Trp - X_3, wherein:

X_4 is  , H, $COCH_3$ or absent;

X_5 is His, H or $COCH_3$; and

X_3 is  , NH_2 or OH;

wherein Y^1 and Y^2 are independently a hydrogen atom, or are taken together to form a carbonyl or thiocarbonyl;

R_1 is H, $COCH_3$, C_2H_5, CH_2Ph, COPh, $COOCH_2Ph$, COO-t-butyl, CH_2CO-(polyethylene glycol) or A;

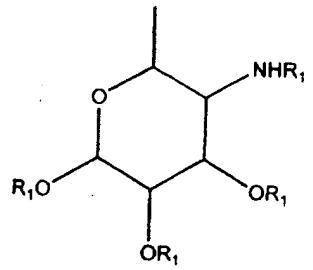
R_2 is H, C_2H_5, CH_2Ph or $COCH_3$;

R_4 is $(CH_2)_m$-$CONH_2$, $(CH_2)_m$-$CONHR_1$ or $(CH_2)_m$-CONHA;

R_5 is OH, OR_3, NH_2, SH, $NHCH_3$, $NHCH_2Ph$ or A; and

R_6 is H or R_3;

and wherein "Ph" is C_6H_5, "m" is 1, 2 or 3, "n" is 0, 1, 2 or 3, and "A" is a carbohydrate having the general formula:



15. The composition of claim 14, wherein the amino terminus of said CRA is modified by acetylation.

16. The composition of claim 14, wherein the carboxy terminus of said CRA is modified by amidation.

17. The composition of claim 14, wherein R_1 is selected from the group consisting of C_2H_5 and CH_2Ph and wherein R_2 is selected from the group consisting of H and $COCH_3$.

18. The composition of claim 14, wherein R_1 and R_2 are the same moiety, said moiety selected from the group consisting of H, C_2H_5 and CH_2Ph.

19. The composition of claim 14, wherein R_5 is covalently bound to X_4, said covalent bond forming a cyclic peptide.

20. The composition of claim 14, wherein said CRA has the structure: His-(D)Phe-Arg-(D)-Trp-Gly.

21. The composition of claim 20, wherein the amino terminus of said CRA is acetylated and the carboxy terminus of said CRA is modified by amidation.

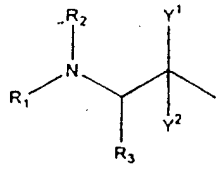
AMENDED CLAIMS

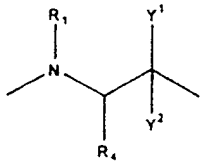
[received by the International Bureau on 5 February 1998 (05.02.98); original claims 1-4 cancelled; original claims 5,7-9,12-14,16-18,20 and 21 amended; new claims 22-28 added; remaining claims unchanged (6 pages)]

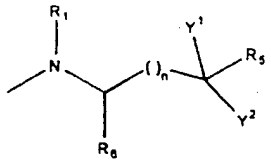
5. A composition of matter, comprising a microemulsion, an adjuvant and a cytokine regulatory agent (CRA) having the structure:

X_1 - X_2 - His - (D)Phe - Arg - (D)Trp - X_3,

wherein:

X_1 is  , H or $COCH_3$;

X_2 is  ; and

X_3 is  or R_5;

wherein Y^1 and Y^2 are independently a hydrogen atom, or are taken together to form a carbonyl or thiocarbonyl;

R_1 is H, $COCH_3$, C_2H_5, CH_2Ph, COPh, $COOCH_2Ph$, COO-t-butyl, CH_2CO-(polyethylene glycol) or A;

R_2 is H, C_2H_5, CH_2Ph or $COCH_3$;

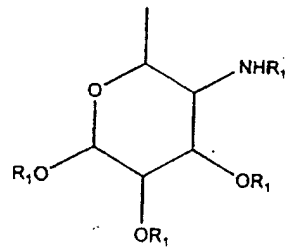
R_3 is a linear alkyl group having 1 to 6 carbon atoms or a cyclic or branched alkyl group having 3 to 6 carbon atoms;

R_4 is $(CH_2)_m$-$CONH_2$, $(CH_2)_m$-$CONHR_1$ or $(CH_2)_m$-CONHA;

R_5 is OH, OR_3, NH_2, SH, $NHCH_3$, $NHCH_2Ph$ or A; and

R_6 is H or R_3;

and wherein "Ph" is C_6H_5, "m" is 1, 2 or 3, "n" is 0, 1, 2 or 3, and "A" is a carbohydrate having the general formula:



6. The composition of claim 5, wherein the amino terminus of said CRA is modified by acetylation.

7. The composition of claim 5, wherein the carboxyl terminus of said CRA is modified by amidation.

8. The composition of claim 5, wherein R_1 is selected from the group consisting of H, C_2H_5 and CH_2Ph.

9. The composition of claim 5, wherein R_1 and R_2 are each H.

10. The composition of claim 5, wherein X_1 is selected from the group consisting of norleucine, norvaline, leucine or isoleucine.

11. The composition of claim 5, wherein R_5 is covalently bound to X_1, said covalent bond forming a cyclic peptide.

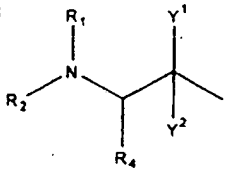
12. The composition of claim 4, wherein said CRA is

Gln - His - (D)Phe - Arg - (D)Trp - Gly-NH_2.

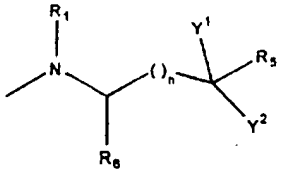
13. The composition of claim 4, wherein said CRA is Ac-Nle - Gln - His - (D)Phe - Arg - (D)Trp - Gly-NH_2.

14. A composition of matter, comprising a microemulsion, an adjuvant and a cytokine regulatory agent (CRA) having the structure:

X_4 - X_5 - (D)Phe - Arg - (D)Trp - X_3, wherein:

X_4 is  , H, $COCH_3$ or absent;

X_5 is His, H or $COCH_3$; and

X_3 is  , NH_2 or OH;

wherein Y^1 and Y^2 are independently a hydrogen atom, or are taken together to form a carbonyl or thiocarbonyl;

R_1 is H, $COCH_3$, C_2H_5, CH_2Ph, COPh, $COOCH_2Ph$, COO-t-butyl, CH_2CO-(polyethylene glycol) or A;

R_2 is H, C_2H_5, CH_2Ph or $COCH_3$;

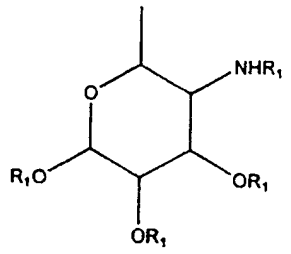
R_3 is a linear alkyl group having 1 to 6 carbon atoms or a cyclic or branched alkyl group having 3 to 6 carbon atoms;

R_4 is $(CH_2)_m$-$CONH_2$, $(CH_2)_m$-$CONHR_1$ or $(CH_2)_m$-CONHA;

R_5 is OH, OR_3, NH_2, SH, $NHCH_3$, $NHCH_2Ph$ or A; and

R_6 is H or R_3;

and wherein "Ph" is C_6H_5, "m" is 1, 2 or 3, "n" is 0, 1, 2 or 3, and "A" is a carbohydrate having the general formula:



15. The composition of claim 14, wherein the amino terminus of said CRA is modified by acetylation.

16. The composition of claim 14, wherein the carboxyl terminus of said CRA is modified by amidation.

17. The composition of claim 14, wherein R_1 is selected from the group consisting of H, C_2H_5 and CH_2Ph.

18. The composition of claim 14, wherein R_1 and R_2 are each H.

19. The composition of claim 14, wherein R_5 is covalently bound to X_4, said covalent bond forming a cyclic peptide.

20. The composition of claim 14, wherein said CRA has the structure: His-(D)Phe-Arg-(D)Trp-Gly.

21. The composition of claim 20, wherein the amino terminus of said CRA is acetylated and the carboxyl terminus of said CRA is modified by amidation.

22. The composition of claim 5 or 14, wherein the adjuvant is salicylic acid.

23. The composition of claim 5 or 14, wherein the composition is in a form for oral administration.

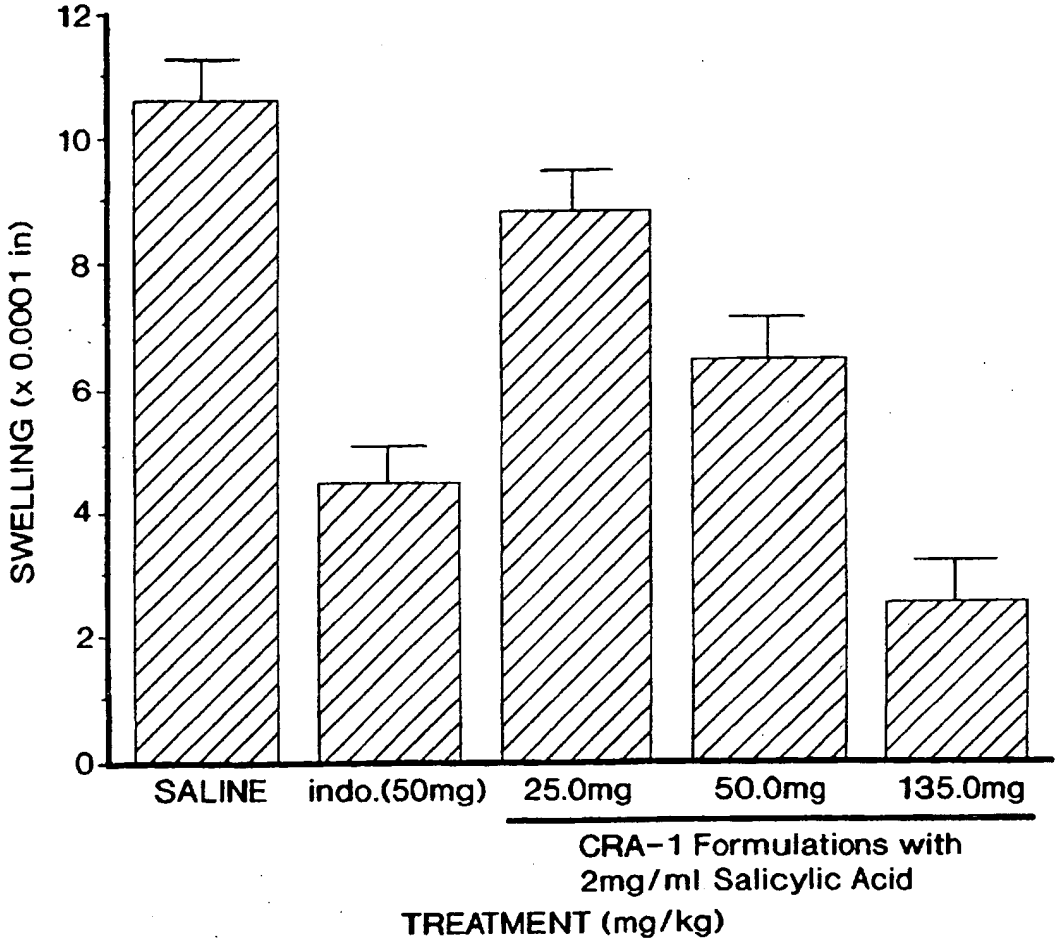
24. The composition of claim 5 or 14, wherein the microemulsion comprises a formulation of an oil and a surfactant.

25. The composition of claim 24, wherein the oil is medium-chain triglycerides.

26. The composition of claim 24, wherein the surfactant is polyglyceryl oleate FCC.

27. The composition of claim 24, wherein the microemulsion further comprises a cosurfactant.

28. The composition of claim 27, wherein the cosurfactant is saturated polyglycolyzed C_3-C_{10} glycerides.


12
10
8
6
4
2
0
SWELLING (x 0.0001 in)
SALINE
indo.(50mg)
25.0mg
50.0mg
135.0mg
CRA-1 Formulations with
2mg/ml Salicylic Acid
TREATMENT (mg/kg)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/16837

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/00, 31/60, 38/00, 38/02, 38/07, 38/08, 38/12
US CL : 514/2, 8,12, 16, 17, 18, 165

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 8,12, 16, 17, 18, 165

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,553,597 A (LE RIBAULT et al.) 19 November 1985, col. 4, lines 39-58.	1-3
X Y	US 5,444,041 A (OWEN et al.) 22 August 1995, entire document.	1, 3 2, 4-21
Y	US 5,420,109 A (SUTO et al.) 30 May 1995, entire document.	1-21
Y	US 4,464363 A (HIGUCHI et al.) 07 August 1984, entire document.	1-21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
28 OCTOBER 1997

Date of mailing of the international search report
08 DEC 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
GABRIELE E. BUGAISKY
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/16837

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CONSTANTINIDES et al. Formulation and Intestinal Absorption Enhancement Evaluation of Water-in-oil Microemulsions Incorporating Medium-Chain Glycerides. Pharmaceutical Research, October 1994, Vol. 11, No. 10, pages 1385-1390, especially Figures 3 and 4.	1, 4
Y		2, 5-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/16837

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

CAS-STN (files registry, hcaplus, uspatfull, wpids), DIALOG (files medline, biosis, dissertation abstracts, derwent wpi), APS
search terms: microemulsion?, adjuvant?, vaccine?, salicyl?, labrasol, plurol oleique, labrafac, caffeine, hydroquinone, oleate, triglyceride?, cytokine? peptide?, "therapeutic use", therap?, regul? restrain?,
also : chemical structure search